The Role of TLR4 in Diet-Induced Behavioral Sensitization

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ABSTRACT

Neuropathic pain is characterized as a condition in which peripheral neurons are damaged or injured, leading to hypersensitivity and chronic pain. It is suggested that the western diet (WD) contributes to neuronal sensitization, however, the exact cause is unclear. Preliminary studies have shown saturated fatty acids in WD can sensitize other cell types. Toll-4 (TLR4) is a receptor commonly found on immune cells. TLR4 plays a major role in pathogen recognition by recognizing the lipid backbone of gram-negative bacteria. Thus serving as an indicator for the presence of bacteria in humans. The lipid backbone of free fatty acids is similar in composition to the lipid backbone of bacteria. Interestingly, it has been recently discovered that the TLR4 is also located on neurons and direct stimulation of fatty acids could be the reason for this sensitization.

We use mice with TLR4 expressed specifically on peripheral neurons to determine their role in the development of diet-induced behavioral sensitivity. We focused on evoked-pain behaviors of the animals. When comparing mice given normal chow to mice given a high-fat diet, it appears that sensitization did occur. We used two mechanisms to measure sensitization, and these were the use of drugs, Carrageenan and PGE2. Normally, carrageenan elicits a modest and transient response, while a sub-threshold level PGE2 elicits no response. We found that components in the high-fat diet did in fact dramatically elevate the pain response in the carrageenan model, but did not lead to a change with PGE2.

Interestingly, fasting glucose and a Glucose Tolerance Test (GTT) showed the mice were not diabetic. With that information, it could be determined that behavioral sensitization did not occur from diabetes-induced peripheral neuropathy, but by dietary components independent of TLR4-signaling.

Materials & Methods

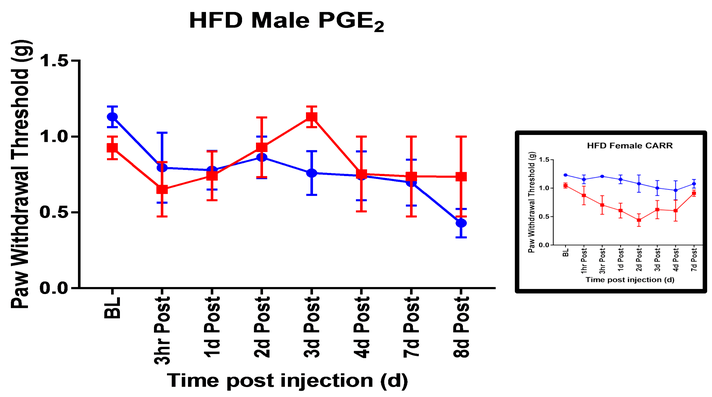
Animals: The Nav1.8 channel Cre is used because the Nav1.8 is found exclusively on nociceptors. A transcriptional blocker (TB) is inserted into any cell containing the TLR4 gene. WT for Cre means the TB is not excised and the mouse is a whole body knock-out for TLR4. Both sexes were used in order to explore the possibility of sex having an impact on sensitization and subsequent behavior of the mice.

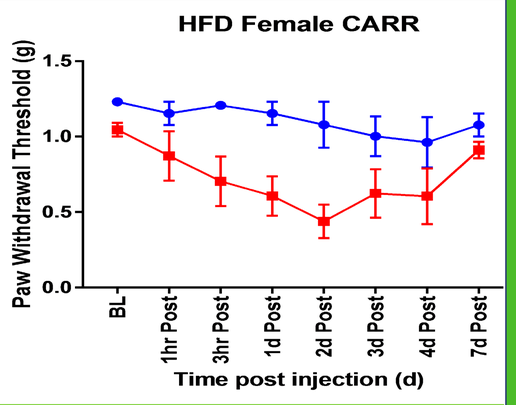
Diet: Mice were given a high-fat diet consisting of 58% calories from fat or normal chow diet (2% calories from fat) *ad libitum*. The animals were weighed once a week starting at six weeks through the course of this experiment.

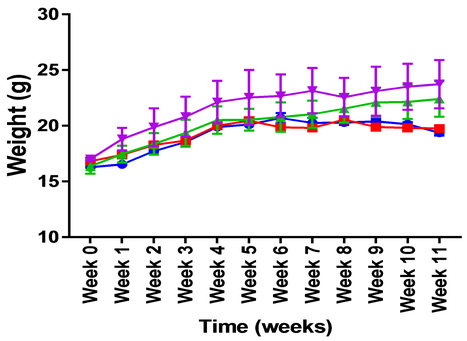
Biochemistry: To prepare for the Glucose Tolerance Test, the mice were fasted overnight. After the fasting period, baseline fasting glucose was measured. Afterwards, each mouse was given an intraperitoneal bolus of glucose (200 mg/kg). Glucose levels were then measured at 20-minute intervals until the levels of all mice had returned to baseline.

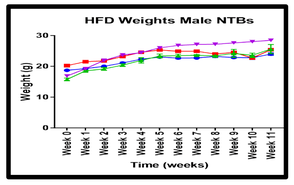
Behavior: For behavior testing, mice were acclimated and habituated to the rack in which they were placed; until the mice were calm. Using calibrated von Frey filaments, the range which mice reacted to was determined. Mice were tested with the filaments in the ipsilateral hind paw to determine the mouse's paw withdrawal threshold. Depending on if the mice responded, thicker or thinner filaments were used. Responses resulted in using thinner filaments, while giving no response resulted in using thicker filaments (otherwise known as the up-down method). This process was repeated to develop a range of filaments in which the mice responded.

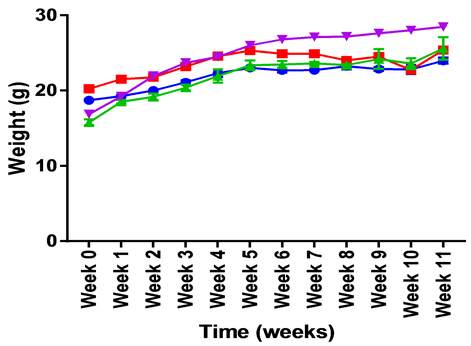
RESULTS

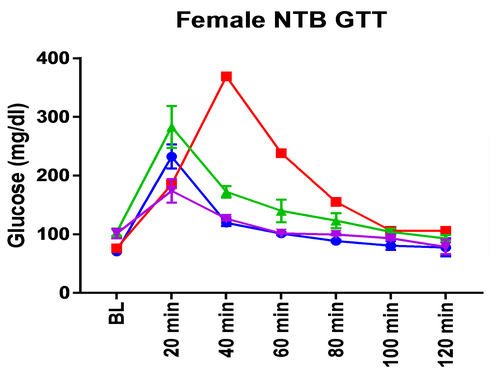


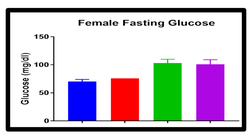


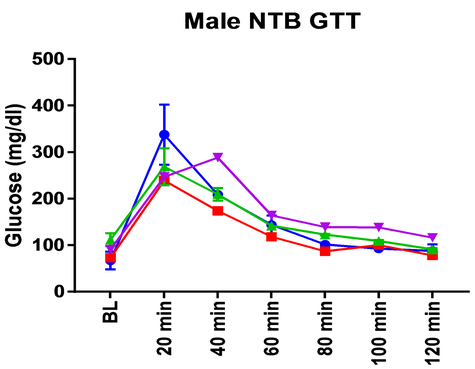


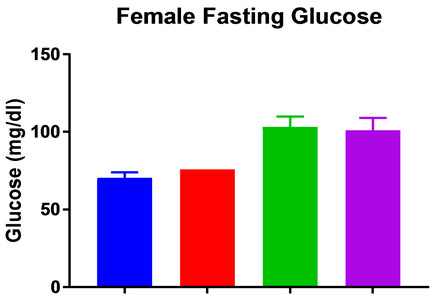


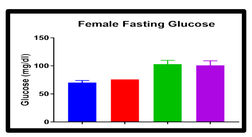


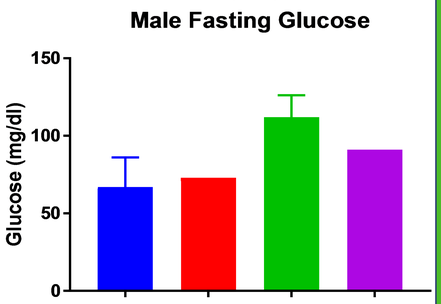












CONCLUSION

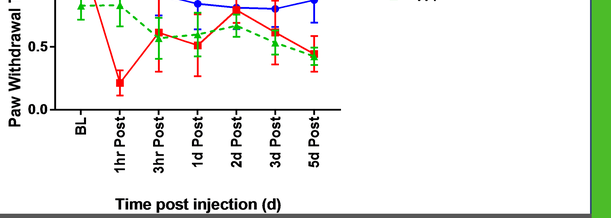
As seen by the GTT results, the glucose levels returned back to or close to baseline levels. This is along with fairly normal fasting glucose levels (~100 mg/dL) is an indicator that the mice were able to properly metabolize glucose. It is possible that the mice were slightly hyperglycemic, but their ability to effectively metabolize glucose showed that the mice were not diabetic.

After being exposed to two drugs, carrageenan and PGE2, data suggested that sensitization did occur, causing an elevated pain response to carrageenan. However, after being tested with PGE2, which does not elicit a response at the sub-threshold level, data showed that there was no change in the sub-threshold level.

FUTURE STUDIES

The mice used in this experiment were placed on high-fat diet for a total of eight weeks. It is known that a prolonged exposure to the diet would in fact make the mice diabetic (12+ weeks). Placing the mice on the high-fat diet for a longer period of time would open up this study to the possible effects or roles that diabetes could play.

Furthermore, experiments to explore specific dietary factors, such as the saturated fatty acid: Palmitic Acid have also been initiated:



REFERENCES

Obrosova IG, IInytska O, Lyzogubov VV, Pavlov IA, Mashtalir N, Nadler JL, Drel VR. 2007. "High-fat diet induced neuropathy of pre-diabetes and obesity." Diabetes.

Chao-Yung Wang, James K. Liao. 2012. "A Mouse Model of Diet-Induced Obesity and Insulin Resistance". Methods Mol Biol.821: 421–433.

Pino-Ribiero, F, Waldiceau, V., Chiu, I. 2017. "Nociceptor Sensory Neuro-Immune Interaction in Pain." Trends in Immunology 38(1).

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